Indonesian Journal of Cancer Chemoprevention, February 2010
ISSN: 2088–0197
e-ISSN: 2355-8989

Antioxidant Activity Test of 2,6-bis-(2'-furilidyn)-Cyclohexanone, ; 2,5-bis-(2'-furilidyn)-Cyclopentanone; 1,5-Difuryl-1,4-pentadien-3-one

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Abstract

Antioxidant is an essential compound to keep man's health due to its function as radical scavenging. Curcumin analog compounds can function as antioxidant (Sardjiman, 2000). The aim of the experiment was to find out the antioxidant activity of 2,.6-bis-(2'-furilidin)-cyclohexanone, 2,.5-bis-(2'furilidin)-cyclopentanone, and 1,.5-difuril-1.4-pentadien-3-one compounds, and the antioxidant activity of each compound against DPPH radical with IC50 parameter as well as the correlation of compounds structure's activities against antioxidant. The antioxidant activity of curcumin analog compounds wereas tested against DPPH free radical. The test was conducted in 5 series of concentrations by adding 4.0 ml test solutions with 1.0 ml DPPH. The antioxidant activity against free radical was measured usingwith spectrophotometer at 517 nm wavelength and determined for the IC50 value. The experiment employed rutin as positive control. The result of the experiment showed that curcumin analog compounds have antioxidant activity with IC50 of rutin, 2,.6-bis-(2'-furilidin)-cyclohexanone, 2,.5-bis-(2'furilidin)-cyclopentanone, and 1,.5-difuril-1.4-pentadien-3-one as follows: 4.93 ppm, 22.73 ppm, 20.67 ppm, and 18.80 ppm respectively. The highest antioxidant activity belonged to 1,.5-difuril-1.4-pentadien-3-one compound which is 18.80 ppm . Correlation of activity structure of the 3 compounds can be seen from the log p parameter and energy space of HOMO-LUMO.

Keywords: Antioxidant, DPPH, Analog Curcumin

INTRODUCTION

Nowadays, there are rapidly-growing research on antioxidant compounds that can deter radical activity which produce various disease. Antioxidant is a compound that can neutralize or confront toxic material (free radical) and prevent cellular oxidation to reduce cell damage. The compound's antioxidant activity can be measured from its ability to catch free radical. Compounds on antioxidant will donate one or more of its electron to alter the free radical to its normal molecule shape and end the damaging process (Caroline 2005; Simanjuntak et al.,. 2004). The effective and relatively safe antioxidant sources are flavonoid, vitamin C, betacarotene, etc. (Pramono et al. 2001). The newly sinthesized curcumin analog compounds have the activity as an antioxidant, anti-inflamation, and antibacterial (Sardjiman 2000).

Curcumin as a radical scavengercatcher is capable of maintaining the integrity of cell's membrane at oxidative degradation caused by oxigent radical and other reactive radicals. Rahmawati (2009) on her previous research successfully sinthesize three curcumin analog compounds, i.e. 2,6-bis-(2'-furilidyn)-cyclohexanone; 2,5-bis-(2'furilidyn)-cyclopentanone; and 1,5-difuril-1,4-pentadien-3-one. The antioxidant activity of these three compounds have not been discovered. The structure of these compounds can be seen at figure 1, 2, and 3.

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One method to determine the antioxidant activity is to use DPPH (1,1- difenil-2-pikrilhidrazil) radical. DPPH compound will give strong absorb for dark violet at 517 nm wavelength. This violet suppression is due to its ability as an antiradical. DPPH method is selected because it is simple, easy, swift, sensitive, and needs only a small amount of sample. Antioxidant compound reacts with DPPH radical through the hidrogen atom donation mechanism and producing the alteration of DPPH's color from violet to yellow (Hernani et al.,. 2005). The free radical catching activity of the compound is expressed with IC50 value which is measured by visible spectrophotometer. IC50 value is defined as substance's effective concentration which can reduce 50% of absorb intensity compared to blank (Caroline 2005).

Base on the above consideration, this research will investigate the antioxidant activity of three curcumin analog compounds, i.e., 2,6-bis-(2'-furilidyn)-cyclohexanone (A compound); 2,5-bis-(2'furilidyn)-cyclopentanone (B compound); and 1,5-difuril-1,4-pentadien-3-one (C compound).

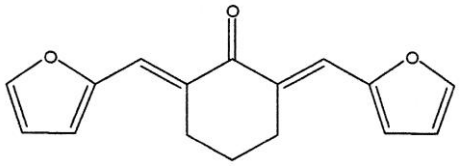

Figure 1. A Compound's structure

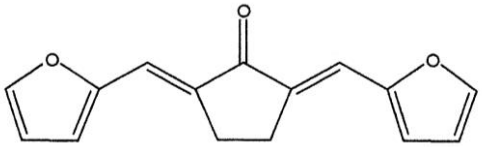

Figure 2. B Compound's structure

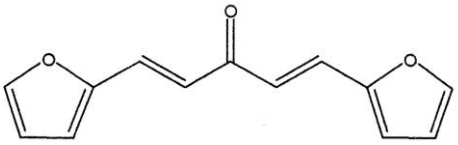

Figure 3. C Compound's structure

METHODS

Mixture preparation.

Preparation of 0.4 mM DPPH in methanol. 0.0158 g DPPH powder was added with methanol in a cylinder measuring glass to reach 100 mL total solution.

Preparation of the experimental solution of A, B, and C compounds. Solve 50 mg of each compound (A, B, and C) into 50 mL solvent to make a concentration of 100ppm of starting solution. Prepare the experimental solution by making 5 series of concentration from each starting solution. These preparations can be seen on table 1.

Preparation of the experimental solution of rutin. Prepare the 100 ppm starting solution by putting 25 mg of rutin in 50 mL scaled glass, and add it with methanol to reach 50 mL total solution. Prepare the experimental solution by making 5 series of concentration from each starting solution. These preparations can be seen on table 2.

Antioxidant activity test

Curcumin analog A, B, C compounds, and the rutin were prepared on 5 series of concentration to get 0.8 – 0.2 absorbance. Take 4.0 ml of each concentration of experimental solution, add it with 1 mL of DPPH reactant solution. Put it down for 30 minutes and observe the absorbance at maximum wavelength. This experimental procedure was done three times. The obtained absorbance was then used in free radical scavenging activity measurement at various concentration. IC_{50} value was calculated from linier regretion curve between probit absorbance prohibition percentage (% prohibition) versus logarithmic of various concentration.

Data analysis

The result of absorbance measurement using UV-Vis spectrophotometer was employed to calculate the percentage of DPPH free radical prohibition. DPPH radical scavenging activity was calculated with prohibition percentage equation (1). Data obtained was then analyzed using linier regretion equation to determine IC_{50}.

RESULTS AND DISCUSSION

IC50 value of each experimental compounds can be seen at table 3. Rutin effectivity (positive control) was different from curcumin analog compounds (A,B, and C) activity. IC50 value of rutin was measured at 4.93 ppm. Based on its IC50 value, C compound has the highest antioxidant activity followed by B and C with IC50 value of 18.80 ppm, 20.67 ppm, and 22.73 ppm , respectively.

Table I. Preparation of A, B, and C compounds series from its starting solution

No	Concentration (ppm)	Starting Solution's Volume (mL)
1	2.5	0.25
2	5	0.5
3	10	1
4	20	2
5	40	4

Table II. Preparation concentration series of rutin compound from its starting solution

No	Concentration (ppm)	Starting Solution's Volume (mL)
1	0.5	0.05
2	1	0.05
3	2.5	0.25
4	5	0.25
5	10	0.5

Table III. IC_{50} value

Replication	IC_{50} value (ppm)			
	Routine	A compound	B compound	C compound
1.	4.99	24.06	21.99	19.96
2.	4.91	22.82	20.51	18.26
3.	4.90	21.33	19.51	18.20
Means±SD/SE	4.93	22.73	20.67	18.80

Table IV. The relation between logP, HOMO, LUMO and IC_{50}

No	Compound	LogP	HOMO	LUMO	ΔE	IC_{50}
1	A	1.87	-8.860	-4.551	4.309	22.73
2	B	1.45	-10.520	-5.687975	4.832025	20.67
3	C	1.25	-10.912	-5.5555	5.3565	18.80

DISCUSSION

Rutin has higher antioxidant activity (more potent) compared to the experimental compounds. Among these three experimental compounds, C compound has the lowest IC50 value, which means C compound has the highest antioxidant activity. This is due to its log p value and its HOMO and LUMO energy.

Chemical compounds reactivity change as its chemical structure change. The chemical structure change will bring the alteration of its biological properties. Whilst using Structure-activity Relationship (SAR) method to study one particular medicine activity, we need certain linked physic-chemical parameters in order to predict the more potent new medicine molecule. Applied physic-chemical parameters were hydrophobic and electronic effect. Observation of A, B, and C compounds on their hydrophobic (log P) and electronic effect (HOMO LUMO) parameters can be seen on table 4. A compound has the highest log P value at 1.87, followed by B and C compounds (1.45 and 1.25, respectively). It means that A compound tends to be at the highest non-polar phase, while C compound tends to be at the lowest non-polar phase. IC50 value shows the smaller its log P value, the higher its antioxidant activity.

HOMO (Highest Occupied Molecular Orbital) and LUMO (Lowest Unoccupied Molecular Orbital) energy are the most popular descriptor in determining various chemical reaction and electronic band gap. HOMO energy is directly link to ionization potential and molecule's weakness property at electrophyl attack. LUMO energy is close related with electron affinity and molecule's weakness property on attack at nucleophyl. The difference between HOMO and LUMO energy (HOMO-LUMO gap) is important at measuring the molecule's stability. Molecule with large HOMO-LUMO gap has a high stability so it has a low reactivity on chemical reaction (Kartrizky et al., 1996). Experimental compound with the largest HOMO-LUMO gap is C compound at 5.3565 followed by B and A compounds (4.832025 and 4.309 respectively). The bigger HOMO-LUMO gap, the more potent its antioxidant activity.

CONCLUSION

From the activity test on the derivate of curcumin analog compounds in this research we can conclude that:

1. All compound 2,6-bis-(2'-furilidyn)-cyclohexanone (A compound); 2,5-bis-(2'furilidyn)-cyclopentanone (B compound); and 1,5-difuril-1,4-pentadien-3-one (C compound), have antioxidant activities.
2. 2,6-bis-(2'-furilidyn)-cyclohexanone (A compound); 2,5-bis-(2'furilidyn)-cyclopentanone (B compound); and 1,5-difuril-1,4-pentadien-3-one (C compound) have IC_{50} value of 22.73 ppm, 20.67 ppm, and 18.80 ppm, respectively. It means that 1,5-difuril-1,4-pentadien-3-one compound is the most active.
3. The antioxidant activity relation of the three compounds is demonstrated by the connection between log P, HOMO, LUMO, and IC50 value, where the lower log P value the higher its antioxidant activity, and the higher HOMO-LUMO gap the higher its antioxidant activity.

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